

Fate and Transport of 17 β -Estradiol in Soil–Water Systems

FRANCIS X. M. CASEY,^{*,†}
 GERALD L. LARSEN,[‡]
 HELDUR HAKK,[‡] AND JIŘÍ ŠIMŮNEK[§]

Department of Soil Science, North Dakota State University, Fargo, North Dakota 58105, Animal Metabolism Unit, Biosciences Research Laboratory, USDA-ARS, Fargo, North Dakota 58105, and George E. Brown, Jr. Salinity Laboratory, USDA-ARS, Riverside, California 92507

Over the past several years, there has been an increase in concern regarding reproductive hormones in the environment. To date, there exists limited research on the fate and transport of these chemicals in the environment. In this study, a series of laboratory batch sorption and miscible-displacement experiments were done using radiolabeled [¹⁴C]17 β -estradiol. The 17 β -estradiol concentrations that were used were similar to those found in manures that are applied to field soils. Equilibrium batch experiments indicated high sorption affinity with correlations to mineral particle size and organic matter content. The sorption affinity appeared to be associated with the surface area and/or the cation-exchange capacity of the soil. The miscible-displacement breakthrough curves indicated chemical nonequilibrium transport, and a single highly polar metabolite was present in the column effluent along with sporadic and trace detections of estradiol. Sorbed to the soil within the column were found 17 β -estradiol, estrone, and trace and sporadic detections of estradiol. Two chemical nonequilibrium, miscible-displacement models were used to describe the column breakthrough curves; one without transformations and the other with transformations. Both models resulted in excellent descriptions of the data, which indicated nonunique solutions and less confidence in the parameter estimates. Nonetheless, the modeling and experimental results implied that degradation/transformation occurred in the sorbed phase and was rapid. Also, both models indicated that sorption was fully kinetic.

Introduction

The presence of low levels of bioactive chemicals in the environment have become a concern to the United States, other countries, and international organizations (e.g., World Health Organization, United Nations). A congressional bill (H.R. 1712) was submitted to the 106th U.S. Congress that proposed to amend the Federal Water Pollution Act to authorize an estrogenic substance screening program. Koplín et al. (1) has recently completed an extensive reconnaissance of surface waters in which 139 streams were sampled across 30 states in the United States. Reproductive hormones were found in approximately 40% of the 139 streams sampled. A

great number of people and wildlife can be impacted by exposure to reproductive hormones, especially since they can produce adverse effects at remarkably low concentrations (<1.0 ng L⁻¹; 2–4). For example, 17 β -estradiol has been found to cause vitellogenin production in male fish at environmental concentrations of 1.0 ng L⁻¹ (2).

Possible exposure to estrogens may come from animal manures that are applied to agricultural fields. Estrogens are naturally produced and excreted by animals, and they are also administered as growth promoters to help in efficiency of feed utilization. When used as such, they are given as benzoate and palmitate esters of estradiol, and the body will hydrolyze them to 17 β -estradiol. On the eastern shore of Maryland, it has been estimated that 200 000 t yr⁻¹ of broiler chicken manure is produced, which contains 30 ng g⁻¹ (30 ppb) of 17 β -estradiol. This is equivalent to about 6 kg of 17 β -estradiol being applied to fields when the manure is used as a fertilizer (5). In 1997, the entire U.S. poultry industry produced over 10 billion kg of broiler litter, 90% of which was applied to crop lands. In a worst case situation, this amount of manure can potentially contain 270 kg of 17 β -estradiol, which, at a 1.0 ng L⁻¹ concentration, has the capacity to contaminate 270 km³ of water.

In an experiment where manure was applied to a field, 17 β -estradiol was found in a nearby free flowing stream at concentrations of 5 ng L⁻¹ (5). In another experiment where manure was applied to a field, the concentrations of 17 β -estradiol in surface runoff reached 150–2300 ng L⁻¹ (6). Recently, Renner (7) reported that hormone adulterated runoff from cattle feedlots could be affecting local fish. Furthermore, Nichols et al. (8), Shore et al. (9), and Peterson et al. (10) have identified 17 β -estradiol (highest concentration of 37.6 ng L⁻¹) in aquifers underlying areas where animal wastes have been applied. Limited studies have been done on the persistence (11–13) and sorption (14, 15) of estrogens in soil and sediment, but little is known about the fate and transport of these chemicals in the environment.

It is essential to understand the fate and transport processes of estrogens in the environment in order to assess their potential impacts on soils, surface water, and groundwater resources. The objectives of this research were to identify the sorption, fate, and mobility of 17 β -estradiol in soil–water systems. These objectives would identify the general effects of soil composition on these parameters by using laboratory batch sorption and miscible-displacement experiments. Also, 17 β -estradiol was chosen for the following reasons: (i) it is potent at low concentrations (<1.0 ng L⁻¹) and has the capability of impacting very large amounts of soil and water resources; (ii) it has the capability of producing other potent metabolites; (iii) it is a prototype chemical for all endocrine disruptors that interact through the estrogen receptor; (iv) it is eliminated (i.e., excreted) by all studied organisms, from cattle and poultry to humans, and therefore it has the potential to be widely distributed in the environment; and (v) little is known about how 17 β -estradiol and its metabolites behave in the environment.

Materials and Methods

The soils that were used were Bearden-silty clay loam, Gardena-clay loam, Glyndon-sandy clay loam, LaDelle-silt loam, and Sioux-loam (taxonomic descriptions provided in Table 1). All these soils, except the Glyndon, were obtained from Ag-Vise Company (Northwood, ND) and were collected in North Dakota and represent a variety of soil textures (Figure 1). Additionally, a medium quartz sand (250–500 μ m) and kaolinite and bentonite clays were used in the batch

* Corresponding author phone: (701)231-8577; fax: (701)231-7861; e-mail: francis.casey@ndsu.nodak.edu.

[†] North Dakota State University.

[‡] USDA-ARS, Fargo.

[§] USDA-ARS, Riverside.

TABLE 1. Soil Fractions Used for Sorption and Miscible-Displacement Experiments, Their Official Soil Series Description, and Organic Matter Content

soil series	taxonomic description	organic matter content (%)	specific surface (m ² g ⁻¹)	pH
Bearden-silty clay loam	fine-silty, mixed, superactive, frigid Aeric Calciaquolls	7.5	175	6.4
Gardena-clay loam	coarse-silty, mixed, superactive, frigid Pachic Hapludolls	5.3	154	6.4
Glyndon-sandy clay loam	coarse-silty, mixed, superactive, frigid Aeric Calciaquolls	3.3	123	
LaDelle-silt loam	fine-silty, mixed, superactive, frigid Cumulic Hapludolls	9.2	151	7.9
Sioux-loam	sandy-skeletal, mixed, frigid Entic Hapludolls	7.5	106	7.8
bentonite		0.0	654	
kaolinite		0.0	43	
sand		0.0		

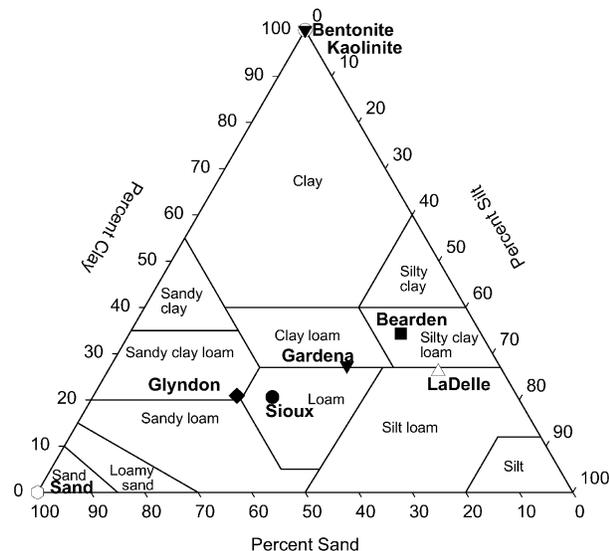


FIGURE 1. United States Department of Agriculture textural triangle showing the mineral particle size distribution of the soils used in this study.

experiments. All the soils were initially dried at 85 °C for 24 h and sieved. Major physical and chemical properties (Table 1) of each soil type were measured at the Soil and Water Environmental Laboratory at North Dakota State University. Specific surface area was measured using the ethylene glycol monoethylene ether method (16).

Statistical analysis was done using SAS (17) to determine the influence of certain soil properties on fate and transport model parameters from the batch and miscible-displacement experiments. The statistical tests that were used were simple correlations, stepwise standard least-squares analysis, and multiple linear regression models. These models were also used in an attempt to distinguish the effects of different soil properties on 17β-estradiol fate and transport parameters.

Batch Sorption Experiments. Batch equilibrium sorption studies were used to identify the sorption of 17β-estradiol to each soil. Soil and water (0.01 M CaCl₂) were added to 10-mL vials in a ratio of 1.6 g:8 mL, respectively. The soil mass for each batch experiment was identical except for the bentonite clay, where only 0.16 g of solid was used because of the extreme swelling of this clay. The batch equilibrium experiments were done using various concentrations of ¹⁴C-radiolabeled 17β-estradiol (American Radiolabeled Chemicals, St. Louis, MO), where the radiolabeled carbon was located in position 4 of the A-ring. The ¹⁴C-spiked 17β-estradiol was added to triplicate vials to create solution concentrations of 0.15, 0.015, and 0.0015 μg mL⁻¹. These concentrations were chosen because they were within the range of 17β-estradiol that has been found in manure applied to agricultural fields (6). The soil–water slurries were agitated by rotation of the vials top to bottom (360°/5 s).

After 48, 96, and 168 h, the bottles were centrifuged at 1700 rpm (380g for 20 min), and triplicate 100-μL aliquots were removed and assayed for radioactivity by liquid scintillation counting using a 1900 CA scintillation counter (Packard, Downers Grove, IL). Thin-layer chromatography (TLC) was used to determine if transformation occurred. The TLC was done using silica gel plates (250 μm; Whatman Lab. Div., Clinton, NJ) with the following solvent systems: (a) methylene chloride:hexane (1:1) and (b) tetrahydrofuran:ethyl acetate:hexane (12.5:12.5:25). [System 2000 Imaging Scanner (Bioscan, Inc., Washington, DC)]. Also, an assay for total ¹⁴C sorbed to soil was done by combustion analysis on a Packard model 307 oxidizer (Downers Grove, IL). Using these methods, the detection limit for 17β-estradiol was 1.5 ppb in terms of water (8 mL) and 7.5 ppb in terms of soil (1.6 g).

After the bottles were centrifuged, the supernatant appeared to be clear, and it was assumed that the measured ¹⁴C was attributed entirely to the dissolved aqueous phase. The relative centrifugal force was calculated much after the experiments were completed, and it was found to be lower than expected, 380g. The lower than expected centrifugal force meant that the ¹⁴C measured in solution may have included solutes attached to colloids. To identify whether some of the measured ¹⁴C in the supernatant was attributed to colloids, an additional batch experiment was performed. This batch experiment was done using the same procedure described earlier; however, after the centrifugation at 380g for 20 min, another ultracentrifugation at 100000g for 1 h was done. After the ultracentrifugation, the supernatant was assayed for ¹⁴C again. If the ¹⁴C decreased in the final assay, then it was assumed that the loss was attributed to colloids suspended in the solution after the first centrifugation.

Batch Sorption Model. Freundlich sorption isotherms were used to describe the batch equilibrium experiments and help identify the effects of various soil fractions on sorption. In the Freundlich sorption isotherm, the concentration of solute adsorbed on the soil matrix (*S*; mg g⁻¹) is related nonlinearly to the aqueous concentration in the soil solution (*C*; mg L⁻³):

$$S = K_d C^n \quad (1)$$

where *K_d* (L g⁻¹) is the Freundlich distribution coefficient and *n* is an empirical constant that controls the deviation from linearity (linear is *n* = 1). A nonlinear, least-squares approximation method (18) was used to obtain the best-fit of eq 1 to the observed data by optimizing the unknown parameters (i.e., *K_d* and *n*). Also, the coefficient of determination (*r*²) was calculated to measure the goodness of the fit.

Miscible-Displacement Experiments. Each soil series (Table 1) was packed into individual columns, and both chloride ion and 17β-estradiol were passed through each column. Table 2 provides the major physical properties of each column. The soils were evenly packed in glass columns

TABLE 2. Soil Column Physical Properties

soil series	mass of dry soil (g)	volumetric water content (cm ³ cm ⁻³)	pore water velocity (cm min ⁻¹)	pore volume (mL)	pulse input (relative PV)
Bearden-silty clay loam	760	0.60	0.35	500	0.08
Gardena-clay loam	699	0.62	0.34	514	0.08
Glyndon-sandy clay loam	1126	0.50	0.34	435	0.09
LaDelle-silt loam	759	0.64	0.35	528	0.08
Sioux-loam	874	0.60	0.38	498	0.08
sand	956	0.57	0.30	471	0.08

(diameter = 8.4 cm, length = 15.2 cm) with stainless steel end caps. Sandwiched between the soil and the end caps were several layer of cheesecloth and a 40 mesh stainless steel screen, which retained the soil in the column. Glass, Teflon, and stainless steel were used in the construction to minimize adsorption to the experimental apparatus.

Each column was slowly wetted, from the bottom up, over a 24-h period using a weak salt solution (0.01 M CaCl₂). This was done to reduce the amount of entrapped air and to maintain soil structure. After the column was wetted, flow was established from the top down using the same 0.01 M CaCl₂ salt solution. Once steady-state pore water velocity (*v*; cm min⁻¹) was achieved, a pulse of chloride ion tracer (0.05 M CaCl₂) was applied and eluted with the 0.01 M CaCl₂ solution. The effluent was fraction collected every 2 min, and conductivity of each fraction was measured using a conductivity meter (Oakton PC 300, Vernon Hills, IL). Table 2 provides the volumetric water content (*θ*; cm³ cm⁻³), pore volume (PV), and *v* of each column experiment. The *v* values measured for each column resulted in residence times within the columns that ranged between 40 and 44 min for the soils and about 50 min for the sand.

Following the chloride ion breakthrough curve experiments, several relative pore volumes (RPVs) of the 0.01 M CaCl₂ solution were flushed through the soil column. Then a pulse of [¹⁴C]17β-estradiol (0.65 μCi, 0.2 mg) was applied to the surface of the soil column in 40 mL of 0.01 M CaCl₂ (Table 2) and eluted with the 0.01 M CaCl₂ solution for at least 7–12 RPVs. The steady-state *v* was essentially constant for both chloride ion and 17β-estradiol experiments (average coefficient of variability < 3%). The column effluent was fraction collected every 2 min, and each fraction (ca. 20 mL) was analyzed for ¹⁴C using the liquid scintillation method described earlier for the batch experiment. Also, the TLC analysis described earlier for the batch experiments was used to determine the presence of metabolites in the column effluent.

Additionally, at the end of the experiment, the distribution of resident ¹⁴C in the column was determined. The soil was extruded from each column in 1-cm increments, dried, and assayed for ¹⁴C by the combustion analysis described earlier. Solution extracts were then obtained from each 1-cm soil increment by sequential elution with toluene, ethyl acetate, and methanol in an accelerated solvent extractor (model 200; Dionex, Sunnyvale, CA). Analysis for metabolites was then done on these extracts using the TLC analysis described for the batch experiments.

Miscible-Displacement Model. The computer program HYDRUS-1D version 2.0 (19) was used to model the miscible-displacement experiments. This program uses an inverse modeling technique to fit the model solution to observed data in order to estimate fate and transport parameters. The inverse modeling approach uses a least-squares optimization routine to obtain the best-fit model solution and does this by iteratively changing model parameters until the best fit is achieved. Also, the chloride ion and 17β-estradiol miscible-displacement experiments were simultaneously analyzed using the method developed by Casey and Šimůnek (20).

This simultaneous analysis of the two solutes leads to more reliable identification of underlying transport processes because of added constraints to the inverse solution (20).

To describe the dynamic fate and transport, a two-site (21, 22), convective–dispersive model with transformation and Freundlich kinetic sorption was considered. The HYDRUS-1D code assumes that solutes can exist in two phases (aqueous and sorbed) and that transformation processes can be different in each phase. The following is the partial differential equation that governs the nonequilibrium chemical transport for a homogeneous system during one-dimensional, steady-state water flow:

$$\theta \frac{\partial C}{\partial t} + \rho_b \frac{\partial S}{\partial t} = \theta v \lambda \frac{\partial^2 C}{\partial x^2} - \theta v \frac{\partial C}{\partial x} - \mu_w \theta C - \mu_s \rho_b S \quad (2)$$

where *t* is time (h), *ρ_b* is soil bulk density (g cm⁻³), *λ* (cm) is the dispersivity, *x* is depth (cm), and *μ_w* and *μ_s* are first-order degradation/transformation rate constants (h⁻¹) for the liquid and sorbed phases, respectively. For a stable nonsorbing solute, such as the chloride ion tracer, *S* = *μ_w* = *μ_s* = 0.

The HYDRUS-1D code may be used to simulate non-equilibrium interactions between aqueous (*C*) and sorbed (*S*) concentrations in the soil–water system. The equilibrium sorption isotherm relating *S* and *C* is described by the Freundlich isotherm (eq 1).

The concept of two-site sorption (21, 22) is implemented in the HYDRUS-1D code to account for possible nonequilibrium adsorption–desorption reactions. In the two-site model, sorption can occur instantaneously on labile exchange sites (i.e., type-1 sites signified by *S^e*) or kinetically on the remaining exchange sites (i.e., type-2 sites signified by *S^k*). The mass balance equations for the type-2 sites in the presence of degradation/transformation is given by

$$S = S^e + S^k \quad (3)$$

$$S^e = fK_d C^n \quad (4)$$

$$\frac{\partial S^k}{\partial t} = \omega [(1 - f)K_d C^n - S^k] - \mu_s S^k \quad (5)$$

where *ω* is the first-order kinetic sorption coefficient (h⁻¹) and *f* is the fraction of exchange sites that are in equilibrium with the solution phase (–).

An alternative model was also considered that included transformations or production of the various 17β-estradiol metabolites. Recent studies have indicated that 17β-estradiol undergoes rapid transformation in agricultural soils (11). Also, our results from the TLC data indicated that transformation occurred. The chemical nonequilibrium, convective–dispersive model that was considered includes transformation in both aqueous and dissolved phases and is governed by the following partial differential equations:

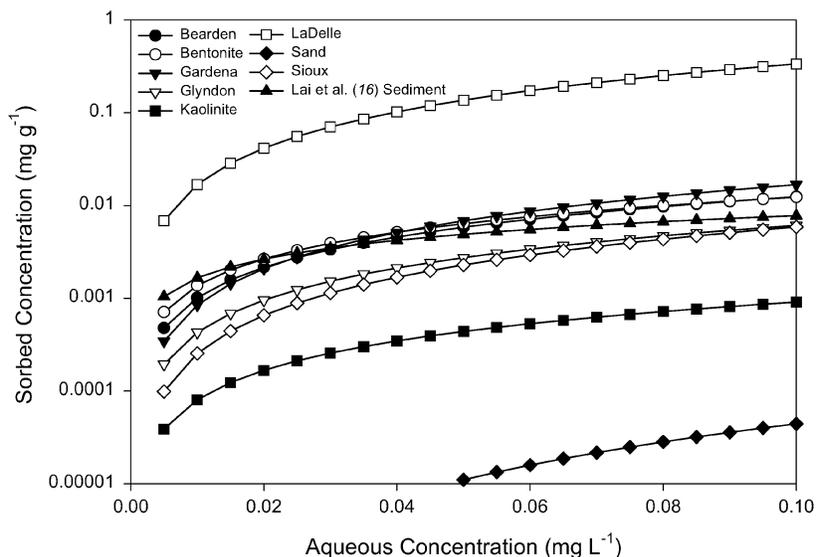


FIGURE 2. Freundlich isotherms obtained from the equilibrium batch experiments from this study as compared with isotherm determined for sediment (20).

$$\theta \frac{\partial C_1}{\partial t} + \rho_b \frac{\partial S_1}{\partial t} = \theta v \lambda \frac{\partial^2 C_1}{\partial x^2} - \theta v \frac{\partial C_1}{\partial x} - (\mu_w + \mu'_w) \theta C_1 - (\mu_s + \mu'_s) \rho_b S_1 \quad (6)$$

$$\theta \frac{\partial C_2}{\partial t} + \rho_b \frac{\partial S_2}{\partial t} = \theta v \lambda \frac{\partial^2 C_2}{\partial x^2} - \theta v \frac{\partial C_2}{\partial x} + \mu'_w \theta C_1 + \mu'_s \rho_b S_1 \quad (7)$$

where μ'_w and μ'_s are first-order transformation rate constants providing mass connection between 17 β -estradiol and its metabolites. The first solute species (C_1 and S_1) can represent 17 β -estradiol that is transformed into a second, mobile species (C_2 and S_2), which was unidentified by the TLC analysis but found to have high polarity. The first species 17 β -estradiol is also degraded/transformed (μ_w and μ_s) into a third species (metabolite), which was identified as estrone. However, estrone was not followed further by the model, and it was assumed that it was strongly adsorbed to the soil. Additionally, estriol was detected in the sorbed and aqueous phases, but these detections were sporadic and insignificant; therefore, it also was not followed by the model. Linear sorption (not Freundlich) was considered in this case because of the model's complexity. Also, two-site sorption with labile and kinetic sorption sites was considered where the mass balance equations for the sorption sites are given by eqs 3–5.

Results and Discussion

Batch Experiments. Measurement of 17 β -estradiol at such low experimental concentrations was complicated by transformations and by possible colloidal suspensions. After 48 h, the batch experiments for 17 β -estradiol appeared to be at equilibrium (i.e., there was little difference in supernatant ^{14}C counts between 48 and 169 h). However, the TLC analysis indicated that there were appreciable amounts of metabolite present after 169 h. The parent compound, 17 β -estradiol, only accounted for approximately 50–70% of the ^{14}C at the 169-h sample time. Additionally, the amount of ^{14}C present in the supernatant decreased by approximately 20% after the ultracentrifugation experiment (100000g for 1 h), which suggested that some ^{14}C was found on colloids suspended in the supernatant. This result indicated the contribution that colloid facilitated transport may have on the fate and transport of 17 β -estradiol.

During the first 48 h, the 17 β -estradiol sorption rates ranged from 0.002 $\mu\text{g g}^{-1} \text{h}^{-1}$ for the sand to 0.112 $\mu\text{g g}^{-1} \text{h}^{-1}$

TABLE 3. Freundlich Sorption Isotherm Parameters and Coefficient of Determination of Isotherm Fit

soil	K_d (L g^{-1})	n	r^2
Bearden-silty clay loam	0.151	1.088	1.00
Gardena-clay loam	0.332	1.297	1.00
Glyndon-sandy clay loam	0.086	1.151	1.00
LaDelle-silt loam	6.670	1.299	1.00
Sioux-loam	0.135	1.363	1.00
bentonite	0.110	0.954	1.00
kaolinite	0.010	1.053	1.00
sand	0.004	2.000	1.00

for the bentonite clay. These 17 β -estradiol sorption rates were similar to sorption rates from river and estuary sediments (0.07–0.37 $\mu\text{g g}^{-1} \text{h}^{-1}$ calculated at 1–5 h) measured by Lai et al. (15).

Freundlich Parameters. The Freundlich isotherm parameters (Table 3) were determined from the 48-h concentrations because of the larger amounts of metabolites found at 168-h sample times. Also, the concentrations did not exclude any possible contributions from colloids in suspension. Lai et al. (15) reported a K_d for 17 β -estradiol of 0.036 L g^{-1} for the sediments, which fell within the lower range of values found in this study (Table 3), but their n value ($n = 0.67$) was lower than any found in this study (Table 3). Although the parameters did not completely agree, the isotherms calculated from the Lai et al. (15) parameters compared well with the isotherms in this study (Figure 2). The difference between the isotherm parameters may be attributed to several differences between studies, including particle size distributions, organic matter content, and suspended colloids. Additionally, Lai et al. (15) used an earlier time, 1 h, to calculate isotherm parameters as compared with the time used in this study, 48 h. At 1 h, the 17 β -estradiol may not have reached an equilibrium sorption concentration, which could result in lower K_d values. On the other hand, transformations may occur at longer times (as used in this study), which may affect the sorption parameter estimates. Except for the bentonite clay, all of the n values from this study were > 1.0 (Table 3), which indicated that sorption site availability did not approach a limit as it did for the sediment study (15). This meant that there was little competition for exchange sites by estrogens.

Batch Sorption Correlations. Correlations between Freundlich parameters and soil fractions were not made

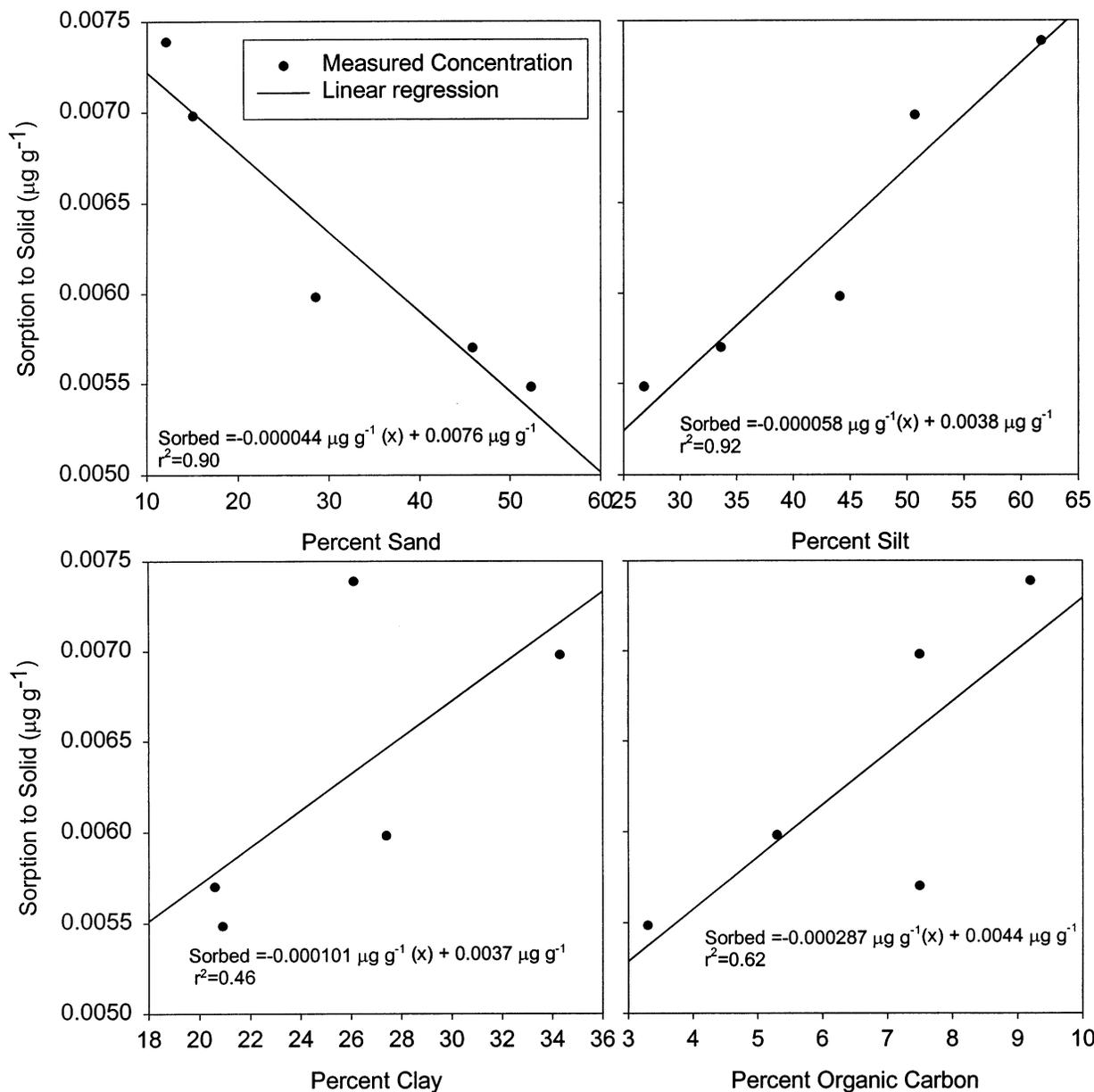


FIGURE 3. Correlations between sorbed estrogen (initial concentration was 0.0015 mg L^{-1} in 8 mL of water and 1.6 g of soil and shaken for 48 h), particle sizes, and organic matter content from the batch experiments.

because the isotherms were nonlinear (i.e., $n \neq 1$). Instead correlations of sorption to various soil fractions were determined for the initial aqueous concentrations of 0.0015 mg L^{-1} in 8 mL of water and 1.6 g of soil and shaken for 48 h. A general correlation between sorption and particle size was found (Figure 3). These correlations reflect a relation between sorption and surface area where specific surface area (Table 1) was highly correlated ($r^2 = 0.92$) to sorption. However, these correlations to particle size may also reflect a sorption mechanism governed by interactions between surface ion-exchange sites and charged or polar solutes. Lai et al. (15) found estrogen sorption to iron oxides and suggest an ion exchange mechanism where polar, phenolate estrogens bind to charged iron oxides. Cation exchange complexes are associated with clay minerals and organic matter and can result in the sorption of polar compounds, such as phenolates. This sorption mechanism may partially explain the difference in the sorption affinity between bentonite (high affinity) and kaolinite (low affinity) clays as well as the soils with the taxonomic designation of "superactive" (Table 1).

Kaolinite has a low specific surface area (Table 1) and a low cation-exchange capacity (CEC) that typically ranges between 3 and 15 cmol kg^{-1} . Bentonite has a high specific surface area (Table 1) and is mainly composed of smectite, which has a high CEC of $100\text{--}150 \text{ cmol kg}^{-1}$. The "superactive" taxonomic designation (Table 1) of the Bearden, Gardena, Glyndon, and LaDelle soils denotes a high CEC, and all these soils have higher isotherms than the nonsuperactive Sioux soil (Figure 2). These results indicate that clay minerals will likely influence sorption, but further experiments are needed to elucidate the binding mechanism of estrogens in natural soils.

Last, there appeared to be a strong correlation between sorption and silt content ($r^2 = 0.92$) (Figure 3); however, this correlation was confounded because silt was also correlated to the organic matter content ($r^2 = 0.68$). The statistical model used was unable to significantly distinguish the effects of organic matter from the effects of silt content on sorption. It would intuitively seem that the organic matter was the cause of this correlation and that the silt correlation was

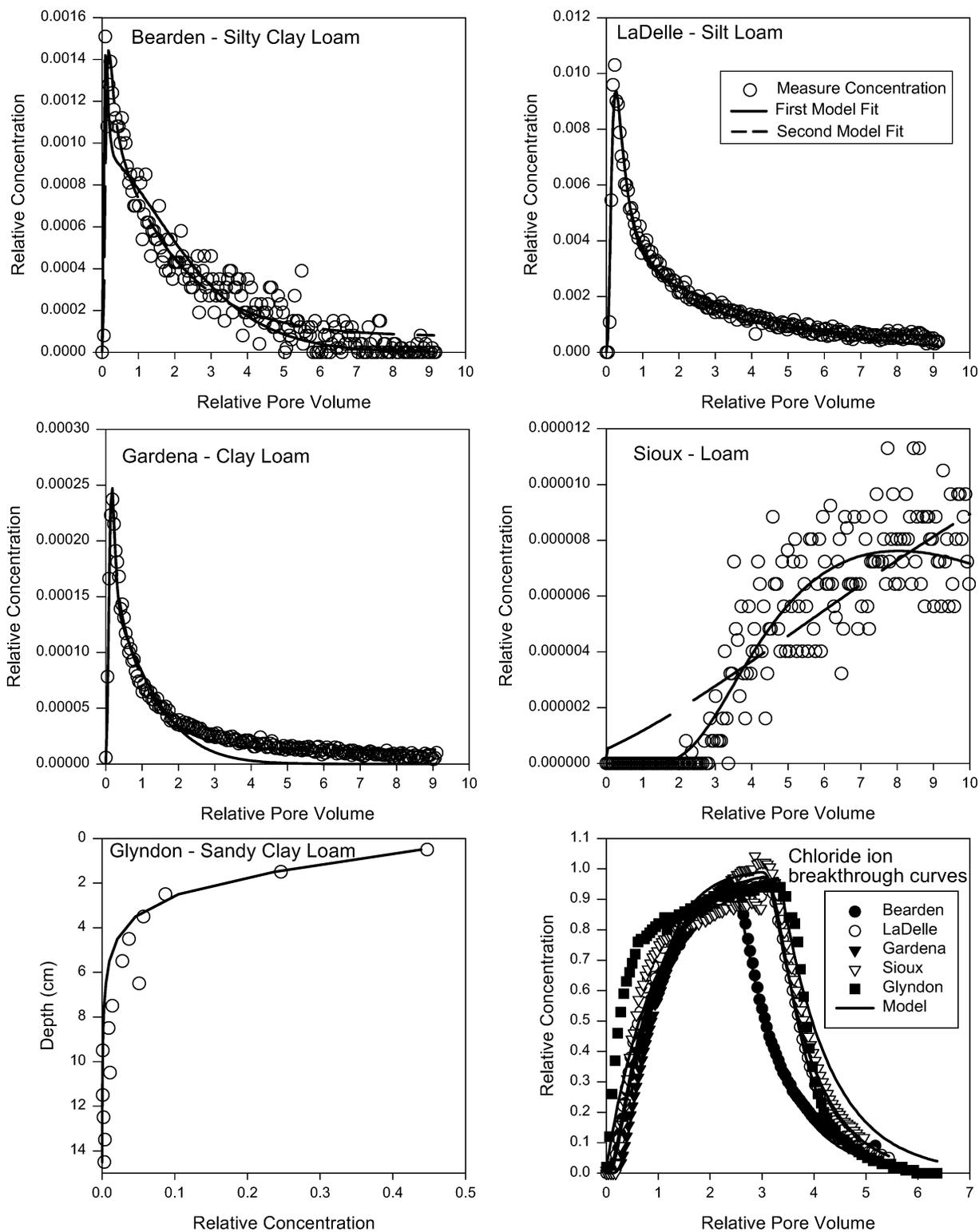


FIGURE 4. 17β -Estradiol column breakthrough curves and corresponding model fits for each soil. The model schematic for the first model (eqs 2–5) and the second model (eqs 6 and 7) are provided in Figure 5. The Glyndon series soil shows the 17β -estradiol distribution within the soil column because there was no effluent breakthrough. Also presented are chloride ion breakthrough curves that were simultaneously modeled with the 17β -estradiol.

coincidental, because silt is relatively inert. Lai et al. (15) also found a strong correlation between total organic carbon, but only weak correlations with particle size distribution.

Miscible-Displacement Experiments. For all the soils, very little or no (i.e., Glyndon-sandy clay loam) ^{14}C was present in the column effluent (Figure 4). Although ^{14}C was not present

in the Glyndon column effluent, there was significant redistribution throughout the column profile (Figure 4). The mass balance of ^{14}C (Table 4) recovered from within the Glyndon soil column was -100% . The TLC analyses indicated that 17β -estradiol was not present in any of the effluents of any soil columns, which suggested little or no aqueous or

TABLE 4. Miscible-Displacement Parameter Estimates for the First Model (Eqs 2–5; Figure 5) with 95% Confidence Interval (in Parentheses)^a

soil	λ (cm)	f	K_d (L g ⁻¹)	n	μ_w (h ⁻¹)	μ_s (h ⁻¹)	ω (h ⁻¹)	r^2	mass recovery ^b (%)
Bearden-silty clay loam	12.556 (0.902)	0	0.769 (2.945)	1.637 (0.494)	0.001 (3.372)	1.494 (0.542)	0.615 (0.802)	0.998	3.2
Gardena-clay loam	10.339 (0.942)	0.008 (0.015)	0.542 (2.590)	1.346 (0.445)	0.001 (9.524)	2.800 (1.009)	0.526 (1.002)	0.997	0.4
Glyndon-sandy clay loam	3.615 (0.514)	0	1.614 (8.940)	1.394 (1.250)	0	0	1.173 (1.781)	0.985	0.0 (99.6)
LaDelle-silt loam	9.062 (1.465)	0.003 (0.004)	1.043 (1.925)	1.873 (0.697)	0.001 (3.739)	0.480 (0.929)	0.152 (0.134)	0.989	20.8
Sioux-loam	61.997 (1238.397)	0.527 (36.728)	0.265 (19.438)	0.984 (0.272)	4.544 (28225.544)	0.284 (44.994)	0.664 (47.231)	0.966	0.1 (75.8)

^a Also present are coefficients of determination of model fit and the mass recovery of ¹⁴C. ^b Mass recovery represents the amount of solute recovered in the column effluent. Values inside parentheses in this column represent the mass recovered from within the soil.

colloidal transport of this highly sorbed chemical. Rather, an unidentified high-polarity metabolite was found in the effluent along with sporadic and trace detections of estriol. Bolton et al. (23) suggest a metabolic pathway where 17 β -estradiol is oxidized to estrone and then further to a more polar semiquinone or quinone. Quinones and semiquinones can cause oxidative damage to DNA and can be ionized (i.e., have a negative charge), which would make them water-soluble and very mobile in soil. An ionized compound could possibly explain the early arrival of ¹⁴C in the column effluent (Figure 4), which appeared nearly at the same time as the chloride ion tracer. It was just as likely that other possible metabolites may have also been present. Layton et al. (13) and Jurgens et al. (24) show transformation of 17 β -estradiol, ultimately leading to mineralization/ring cleavage in activated sludge and river water, respectively.

The TLC analyses of the soil extracts from within each column indicated that the majority of the sorbed ¹⁴C was 17 β -estradiol and another metabolite of lower polarity, which was identified as estrone. Estriol was also detected sporadically in trace amounts in the extracts. These general results indicated that 17 β -estradiol entered the soil column, readily partitioned to the solid phase, and underwent rapid transformation to form at least three metabolites of different polarity. The lower polarity estrone was adsorbed to the soil, and the higher polarity metabolites were more mobile in the column and more readily transported in the aqueous phase. It was possible that some of the ¹⁴C was redistributed through the column by colloidal transport, especially for the highly sorbed estrone and 17 β -estradiol. However, none of the highly sorbed compounds were present in the effluent, only the high polarity metabolite, which was likely transported in the dissolved aqueous phase.

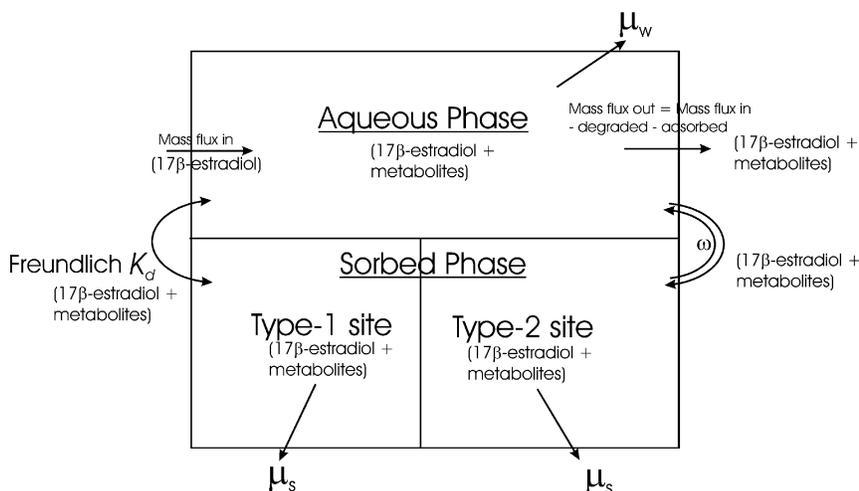
Miscible-Displacement Model Analysis. The breakthrough of ¹⁴C in the column effluents (Figure 4) displayed signs of nonequilibrium transport, which was signified by the long tails or late arrivals of solute. However, the chloride ion tracer was transported through all soil columns as a convective-dispersive process with no sorption or transformation (eq 2 when $S = \mu_s = \mu_w = 0$). The nonequilibrium transport of the ¹⁴C was thus determined to be a result of chemical interactions (e.g., sorption processes, degradation) because there were no strong indications of physical nonequilibrium (i.e., preferential flow) from the chloride ion experiments. The dispersivity (λ) values presented in Table 4 represent a single value that was simultaneously optimized (20) for both chloride and 17 β -estradiol experiments, while assuming ν was constant for both experiments. The λ was the same for the two models that were considered, which added constraints to the other parameter estimates and improved the inverse model uniqueness.

The analysis of the breakthrough curves for the 17 β -estradiol was complicated by the fact that simultaneous transformation and kinetic sorption occurred. The TLC data analyses indicated the presence of three metabolites but were unable to quantify their relative concentrations. Therefore, no quantitative conclusions could be made about the transformation of 17 β -estradiol, which was only found sorbed inside the column and not in the column effluent. It was necessary to make an assumption regarding the transport of the 17 β -estradiol and its metabolites and to consider different types of models to interpret the data.

The two models that were considered were presented in Figure 5. The first model (eqs 2–5) treated the total ¹⁴C as a single species that underwent transformation in the aqueous and sorbed phases. This meant that the identified sorption or transformation rates were identical or represented a lumped value for 17 β -estradiol and its metabolites. The second model (eqs 6 and 7) considered that the 17 β -estradiol entered the soil and was transformed into the unidentified high-polarity metabolite in addition to estrone or estriol. The transformation constants μ_w and μ_s were used for estrone, and μ'_w and μ'_s were used for the polar metabolite. The parameters for the second model were estimated by simultaneously fitting (20) the measured concentrations of 17 β -estradiol and the polar metabolite in the column effluent, where 17 β -estradiol concentrations (C_1 in eqs 6 and 7) were all set to zero and the polar metabolite concentrations (C_2 in eqs 6 and 7) were set to the measured ¹⁴C. The second model could not follow the fate of estrone or estriol, but it could be assumed that they were strongly sorbed to the soil shortly after transformation, which was consistent with the TLC data.

Both the first and the second models had advantages and disadvantages. The first model simultaneously tracked all estrogen compounds, used Freundlich sorption, and had fewer model parameters to estimate. Fewer parameters increase the reliability of their estimates; however, the identical transformation or sorption rates for 17 β -estradiol and its metabolites made this model physicochemically less likely. The second model was physicochemically more realistic, but additional parameters were needed, only linear sorption was considered, and transport of estrone and estriol were not followed after transformation. Both models provided excellent description of the data (indicated by r^2 values in Tables 4 and 5). However, the lack of information about the transformation process, metabolic products, and the high number of fitting parameters resulted in decreased confidence in parameter estimates and decreased uniqueness in the inverse model solution. As a result, the confidence intervals of the parameter estimates (Tables 4 and 5) often spanned the entire spectrum. Additional information about

First Model



Second Model

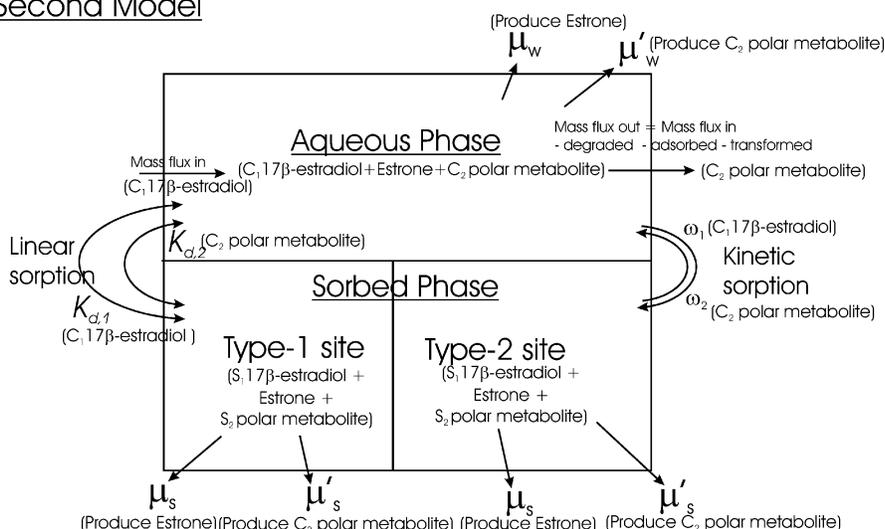


FIGURE 5. Schematics of the two miscible-displacement models that were used to describe the fate and transport of 17 β -estradiol. The arrows indicate mass transfer or transformations of the compounds in the parentheses. Double-headed arrows link compartments that reach equilibrium instantaneously. Also C_1 , C_2 , S_1 , and S_2 were defined in eqs 6 and 7.

TABLE 5. Parameter Estimates for the Second Model (Eqs 6 and 7; Figure 5) with 95% Confidence Interval (in Parentheses)

soil	$K_{d,1}$ ($L\ g^{-1}$)	μ_w (h^{-1})	μ_s (h^{-1})	μ'_w (h^{-1})	μ'_s (h^{-1})	T_1 (h^{-1})	$K_{d,2}$ ($L\ g^{-1}$)	T_2 (h^{-1})	r^2
Bearden-silty clay loam	0.180 (0.920)	0.000 (0.270)	0.418 (0.270)	5.687 (3.080)	0.388 (0.25)	0.292 (1.540)	0.223 (0.110)	0.029 (0.010)	0.931
Gardena-clay loam	0.837 (69.230)	4.069 (5129.890)	1.221 (15.310)	4.949 (5.170)	0.281 (10.61)	0.119 (5.570)	0.412 (0.120)	0.035 (0.010)	0.988
LaDelle-silt loam	0.418 (39.870)	0.001 (0.000)	0.001 (0.600)	34.547 (264.560)	0.766 (4.30)	0.143 (14.510)	0.059 (0.040)	0.057 (0.030)	0.986
Sioux-loam	1.209 (3.000)	0.001 (0.000)	0.299 (1.130)	0.001 (1.680)	0.026 (0.20)	0.333 (0.880)	0.178 (0.590)	0.677 (0.620)	0.818

the relative amounts of the various sorbed metabolites was needed to improve parameter confidence and uniqueness. Nonetheless, the model solutions provided realistic parameter values that could be used to create a hypothesis for the fate and transport of 17 β -estradiol and its metabolites.

The confidence in the modeling was especially low for the Sioux-loam where the effluent concentrations were very low. A complete breakthrough curve was not obtained during the experiment, and measured concentrations were significantly scattered. Consequently, different initial estimates of optimized parameters resulted in widely different estimates

with similar values of the objective function. The optimized parameters for this soil suffered from the largest degree of uncertainty and will not be discussed further.

Sorption. Miscible-displacement sorption coefficients were not held constant to the values obtained from the batch experiments for several reasons. Transformations occurred during the column studies and the metabolite sorption coefficient would not be the same as the batch experiments. Sorption parameters obtained from a column study will be affected by rate-limited mass transfer because of advective transport. Sorption parameters from batch experiments will

not include this rate-limited mass transfer. It should be noted, that the batch sorption values were used as initial guesses for the inverse parameter estimates. This helped to achieve a minimum for the objective function.

The f values for both models were small or zero (Tables 4 and 5) and indicated that sorption was almost fully kinetic with no instantaneous or labile sorption sites (i.e., type-1 sites). All the soil column breakthrough curves modeled with the second model resulted in full kinetic sorption with no type-1 sorption sites, so $f=0$ in each case (Table 5). The only case where labile sorption was significant was for the first model for the Sioux-loam, where model confidence was very low.

Equilibrium sorption estimated by the first and second models compared well with the batch experiments and with other studies. The batch Freundlich parameters (Table 3) fell within the range of values estimated from the first model (Table 4). The linear 17β -estradiol $K_{d,1}$ values of the second model (0.18–1.209 L g⁻¹) compared well with the linearized (where n was forced to equal 1) batch experiment K_d values (0.010–0.322 L g⁻¹). Also, the linearized 17β -estradiol K_d values from the first (0.137–0.661 L g⁻¹) and second (0.180–1.209 L g⁻¹) models were slightly higher but comparable to the linearized K_d value (0.070 L g⁻¹) reported for sediments by Lai et al. (15).

For the second model, the polar metabolite had a similar or slightly lower K_d value as compared to the 17β -estradiol. This indicated that sorption kinetics had a significant role in the mobility of these compounds. The difference in the sorption kinetics was indicated by the difference between ω_1 and ω_2 values for the second model (Table 5). The ω_1 was lower than ω_2 for all soils except the Sioux-loam, where the confidence of the model analysis was low. Using batch kinetic experiments with sediments, Lai et al. (15) shows that 17β -estradiol has more rapid sorption than its metabolite, estriol. The higher ω value of 17β -estradiol would result in a rapid sorption process, which quickly binds it to the soil and makes it less mobile. By separating the ω values between 17β -estradiol (i.e., ω_1) and its metabolite (i.e., ω_2), the second model was able to describe the solute tailing better than the first model (Figure 4). The first model lumped ω for both 17β -estradiol and its metabolites, which resulted in a poorer description of the solute tail. A better description of the data would indicate a more realistic description of the fate and transport processes.

Degradation/Transformations. The first model indicated that nearly all degradation/transformation occurred on the sorbed phase. The second model also indicated a sorbed phase transformation for 17β -estradiol. Previous research (13) on the degradation of 17β -estradiol in biosolids of wastewater treatment facilities provides evidence for sorbed-phase transformations. This earlier research indicates the importance of the amount of 17β -estradiol that remains in the aqueous phase, and how it can decrease the effectiveness of removal by degradation. Furthermore, the sorbed-phase degradation/transformation of 17β -estradiol was consistent with the TLC results where 17β -estradiol, estrone, and trace estriol were the only ¹⁴C found sorbed to the soil and the more oxidized polar metabolite was predominantly found in the aqueous phase.

No efforts were made to isolate biodegradation in this study (i.e., sterilization of the soil). Also, the degradation rates may not have accurately reflected natural rates because biodegradation may have been reduced due to the oven-drying process. Nonetheless, the μ_S values for the first and second models were similar to other studies, which measured degradation in agricultural soils and biosolids. Previous studies have shown that estrogens undergo rapid degradation in agricultural soils (11, 12) and by biosolids in wastewater treatment facilities (13). The 17β -estradiol degradation rates

(0.06–0.12 h⁻¹) reported for agricultural soils (11) fell in the lower range of μ_S values of the first model (0.284–2.800 h⁻¹) and were very similar to the μ_S and μ'_S values (0.001–1.221 h⁻¹) of the second model. Degradation rates (0.252 ± 0.012 h⁻¹) reported for 17β -estradiol in biosolids (13) fell within the range most similar to the μ_S values for the 17β -estradiol of the second model (Table 5). Any differences between μ values from the current column experiments and those reported by earlier studies can be explained by relative uncertainty of the optimized parameter values and by several experimental factors that affect 17β -estradiol degradation/transformation rates. These factors include temperature and water content (11–13). The experiments done in this study were conducted at or near saturation, while the study on persistence in agricultural soils used unsaturated soils (0.13 cm³ cm⁻³). The column experiments of this study contained more soil than the previous studies (11, 13). The greater amount of soil would increase the amount of available sorption sites where degradation may likely occur.

The results from the column experiments and recent studies (11, 13) have indicated rapid degradation/transformation of 17β -estradiol. Colucci et al. (11) found that 17β -estradiol is rapidly transformed into estrone and that estrone persists in the soil for the duration of their experiment (3 d) without further degradation. Other studies have suggested a metabolic oxidation sequence of 17β -estradiol, where estrone and estriol are produced (25), and further transformations can occur to form semiquinones and quinones (23) or possibly result in steroid ring cleavage (13, 24). These studies indicate a sequence of possible transformations that are consistent with the identification of metabolites from our miscible-displacement experiments. However, it was not possible to definitively identify the high-polarity metabolite, which could pose a greater risk (if found to be potent) to surface and subsurface water because of its higher mobility. Additionally, the long-term persistence of estrone in the soil needs to be identified and may lead to impacts on soil and water quality if it is repeatedly applied to the soil, as would occur in a field setting. The preliminary findings from this study nonetheless indicated that no intact 17β -estradiol or identifiable estrogen metabolites could escape from these agricultural soils. It may be concluded that 17β -estradiol-enriched manure could be safely applied to these high organic disturbed soils provided that no bypass flow (e.g., preferential transport) or facilitated (e.g., colloid) transport results in significant depth of transport. The disposal of 17β -estradiol enriched manure would also be precluded in areas where there is significant surface runoff where sorbed 17β -estradiol can be transported in suspension.

Acknowledgments

The authors greatly appreciate the long hours dedicated to this research by the following technical experts: Mrs. Barbara K. Magelky and Mrs. Colleen M. Pfaff. The authors also acknowledge the National Science Foundation for their support of this research.

Literature Cited

- (1) Koplín, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. *Environ. Sci. Technol.* **2002**, *36*, 1202–1211.
- (2) Purdom, C. E.; Hardiman, P. A.; Bye, V. J.; Eno, N. C.; Tyler, C. R.; Sumpter, J. P. *Chem. Ecol.* **1994**, *8*, 275–285.
- (3) Routledge, E. J.; Sheahan, D.; Desbrow, C.; Sumpter, J. P.; Waldock, M. *Environ. Sci. Technol.* **1998**, *32*, 1559–1565.
- (4) Baronti, C.; Curini, R.; D'Ascenzo, G.; Di Corcia, A.; Gentili, A.; Samperi, R. *Environ. Sci. Technol.* **2000**, *34*, 5059–5066.
- (5) Shore, L. S.; Shemesh, M.; Cohen, R. *Aust. Vet. J.* **1998**, *65*, 68.
- (6) Finlay-Moore, O.; Hartel, P. G.; Cabrera, M. L. *J. Environ. Qual.* **2000**, *29*, 1604–1611.

- (7) Renner, R. *Environ. Sci. Technol.* **2002**, *36*, 194A–197A.
- (8) Nichols, D. J.; Daniel, T. C.; Edwards, D. R.; Moore, P. A.; Pote, D. H. *J. Soil Water Conserv.* **1998**, *53*, 74–77.
- (9) Shore, L. S.; Correll, D. L.; Chakraborty, P. K. *Relationship of Fertilization with Chicken Manure and Concentrations of Estrogens in Small Streams; Animal Waste and the Land-Water Interface*: Boca Raton, FL, 1995; pp 155–162.
- (10) Peterson E. W.; Davis, R. K.; Orndorff, H. A. *J. Environ. Qual.* **2000**, *29*, 826–834.
- (11) Colucci, M. S.; Bork, H.; Topp, E. *J. Environ. Qual.* **2001**, *30*, 2070–2076.
- (12) Colucci, M. S.; Topp, E. *J. Environ. Qual.* **2001**, *30*, 2077–2080.
- (13) Layton, A. C.; Gregory, B. W.; Seward, J. R.; Schultz, T. W.; Sayler, G. S. *Environ. Sci. Technol.* **2000**, *34*, 3925–3931.
- (14) Jurgens, M. D.; Johnson, A. C.; Williams, R. J. *Fate and Behavior of Steroid Oestrogens in Rivers; Scoping Study R&D Technical Report P161*; Environment Agency: London, 1999.
- (15) Lai, K. M.; Johnson, K. L.; Scrimshaw, M. D.; Lester, J. N. *Environ. Sci. Technol.* **2000**, *34*, 3890–3894.
- (16) Cihacek, L. J.; Bremner, J. M. *Soil Sci. Soc. Am. J.* **1979**, *43*, 821–822.
- (17) SAS Institute Inc. *SAS Online Doc, version 8*; SAS Institute Inc.: Cary, NC, 2000.
- (18) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Recipes: The Art of Scientific Computing*, 2nd ed.; Cambridge University Press: New York, 1992.
- (19) Šimůnek, J.; Šejna, M.; van Genuchten, M. Th. *The HYDRUS-1D software package for simulating the one-dimensional movement of water, heat, and multiple solutes in variably saturated media*, Version 2.0; IGWMC–TPS–70; International Ground Water Modeling Center, Colorado School of Mines: Golden, CO, 1998; 202 pp.
- (20) Casey, F. X. M.; Šimůnek, J. *J. Environ. Qual.* **2001**, *30*, 1354–1360.
- (21) Selim, H. M.; Davidson, J. M.; Rao, P. S. C. *Soil Sci. Soc. Am. J.* **1977**, *41*, 3–10.
- (22) van Genuchten, M. Th.; Wagenet, R. J. *Soil. Sci. Soc. Am. J.* **1989**, *53*, 1303–1310.
- (23) Bolton, J. L.; Pisha, E.; Zhang, F.; Shengxiang, Q. *Chem. Res. Toxicol.* **1998**, *11*, 1113–1127.
- (24) Jurgens, M. D.; Holthaus, K. I. E.; Johnson, A. C.; Smith, J. J. L.; Hetheridge, M.; Williams, R. J. *Environ. Toxicol. Chem.* **2002**, *21*, 480–488.
- (25) Turan, A. *Endocrinically Active Chemicals in the Environment UBA TEXTE 3/96*; Federal Environmental Agency: Frankfurt, Germany, 1996; pp 15–20.

Received for review September 12, 2002. Revised manuscript received March 12, 2003. Accepted March 19, 2003.

ES026153Z